

Disrupted-in-schizophrenia-1 (DISC1) Regulates Endoplasmic Reticulum Calcium Dynamics

Sung Jin Park¹, Jaehoon Jeong¹, Young-Un Park¹, Kyung-Sun Park², Haeryun Lee², Namgyu Lee¹, Sung-Mo Kim¹, Keisuke Kuroda³, Minh Dang Nguyen⁴, Kozo Kaibuchi³ and Sang Ki Park^{1}*

¹Department of Life Sciences, Pohang University of Science and Technology, 790-784, Republic of Korea

²Division of Integrative Biosciences and Biotechnology, Pohang University of Science and Technology, 790-784, Republic of Korea

³Department of Cell Pharmacology, Nagoya University Graduate School of Medicine, Nagoya, Aichi 466-8550, Japan

⁴Hotchkiss Brain Institute, Departments of Clinical Neurosciences, Cell Biology and Anatomy, and Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada T2N 4N1

** Correspondences should be addressed to SKP at skpark@postech.ac.kr.*

Supplementary Information

Supplementary Figure 1. Immunocytochemical analysis of the DISC1 mutant lacking amino acid residues 1-350 (DISC1^{Δ1-350})

(a) Co-immunostaining of Flag-DISC1^{Δ1-350} and GFP-ER (*i*) and a quantitative analysis of co-localization (*ii*) in HEK293 cells (n=6 for DISC1 WT, 4 for DISC1^{Δ1-350}). Scale bar represents 10 μm.

(b) Co-immunostaining of Flag-DISC1 and GFP-EXOC1 (*i*), Flag-DISC1^{Δ1-350} and GFP-EXOC1 (*ii*), a quantitative analysis of co-localization (*iii*) in HEK293 cells (n=4 for DISC1 WT, 3 for DISC1^{Δ1-350}). Scale bar represents 10 μm.

Supplementary Figure 2. Multiple interaction interface of EXOC1 for DISC1

(a) Schematic mapped domains of EXOC1. (PH; pleckstrin homology, CC; coiled-coil, HB; helical bindles)

(b) Co-immunoprecipitation of GFP-EXOC1 fragments with Flag-DISC1 in HEK293 cells.

Supplementary Figure 3. Characterization of shRNA constructs specific for DISC1 and EXOC1

RNAs were acquired with the RNA preparation kit (SolGent). Complementary DNAs were produced from isolated RNAs with the Superscript III kit (Invitrogen), and the PCR bands were subjected to statistical analyses.

Error bars represent means ±SEM. *, $P < 0.05$, **, $P < 0.01$ (two-tailed *t*-test)

(a) PCR bands of endogenous DISC1 (*i*) and Flag-DISC1 protein bands (*ii*) from control shRNA-, DISC1 shRNA1- or shRNA2-transfected HEK293 cells. DISC1 shRNA2 was mainly used for human DISC1 knockdown in HEK293 cells (n=9 for (*i*), 4 for (*ii*)).

(b) PCR bands of endogenous EXOC1 (*i*) and GFP-EXOC1 protein bands (*ii*) from control shRNA- or EXOC1 shRNA-transfected HEK293 cells (n=3 for (*i*), 4 for (*ii*)).

(c) PCR bands of endogenous mEXOC1 (*i*) and GFP-mEXOC1 protein bands (*ii*) from control shRNA- or mEXOC1 shRNA-transfected mouse neuroblastoma CAD cells (n=8 for (*i*), 3 for (*ii*)).

Supplementary Figure 4. PKA-mediated phosphorylation of IP3R1 in EXOC1-knockdown

Phosphorylation of IP3R1 in EXOC1 knockdown HEK293 cells. (n= 6). Error bars represent means ±SEM.

Statistical significance was analyzed by two-tailed *t*-test.

Supplementary Figure 5. Effects of enhanced PKA activity on IP₃R1 phosphorylation by forskolin and ER calcium dynamics

- (a) Up-regulation of IP₃R1 phosphorylation upon treatment with 100 μ M forskolin in HEK293 cells.
- (b) ER calcium response curves under 50 μ M ATP stimulation in 100 μ M forskolin-treated HEK293 cells (i), and statistically analyzed average area (ii) and amplitude (iii) (n=80 for vehicle, 80 for forskolin).

Error bars represent means \pm SEM. ***; $P < 0.001$ (two-tailed *t*-test)

Supplementary Figure 6. IP₃R-dependent ER calcium responses of DISC1 overexpression in EXOC1-knockdown cells and EXOC1 overexpression in DISC1-knockdown cells

- (a) Rescue of abnormal IP₃R-dependent ER calcium dynamics in EXOC1-knockdown cells. Effect of EXOC1 overexpression in DISC1-knockdown cells were analyzed in parallel, Average area (b) and amplitudes (c) of calcium response graph under 50 μ M ATP stimulation were statistically analyzed (n= 79 for control shRNA, 26 for EXOC1 shRNA, 24 for DISC1 shRNA, 27 for EXOC1 shRNA+Flag-DISC1, 24 for EXOC1 shRNA+RFP-EXOC1). Error bars represent means \pm SEM. *; $P < 0.05$, **; $P < 0.01$. ***; $P < 0.001$ (two-tailed *t*-test)

Supplementary Figure 7. ER calcium responses upon blockade of mitochondrial calcium uptake

- (a) IP₃R-dependent ER calcium dynamics upon DISC1 or EXOC1 knockdown in HEK293 cells treated with ruthenium red (10 μ M). Average area (b) and amplitudes (c) of the calcium response graph under 50 μ M ATP were statistically analyzed (n= 44 for control shRNA, 47 for EXOC1 shRNA, 72 for DISC1 shRNA). Error bars represent means \pm SEM. *; $P < 0.05$, **; $P < 0.01$. ***; $P < 0.001$ (two-tailed *t*-test)

Supplementary Figure 8. No significant changes in ER basal calcium level and caffeine-induced ER calcium release in DISC1- or EXOC1-knockdown cells

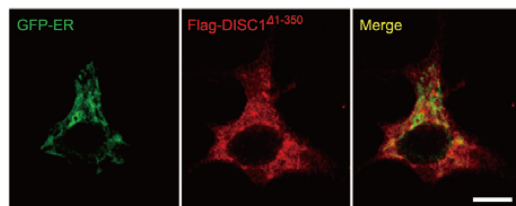
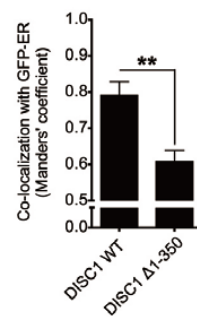
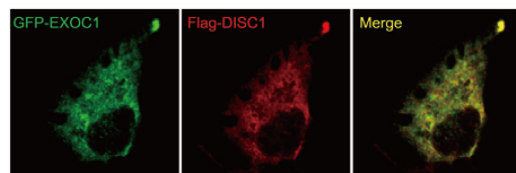
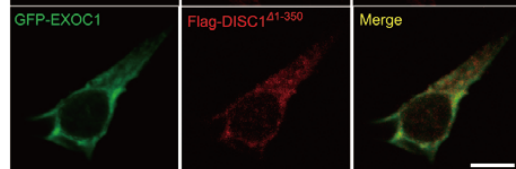
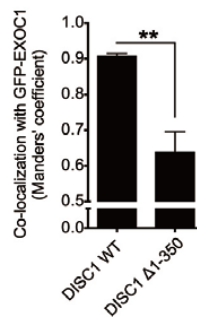
- (a) Co-localization between GFP-ER (green) and ER-aequorin (ER-AEQ, red) in CAD cells (i) and relative ER calcium responses detected by reconstituted ER-AEQ in mDISC1- and mEXOC1-knockdown CAD cells (ii). Amplitudes (iii) were statistically analyzed (n= 10 for control shRNA, 4 for mEXOC1 shRNA, 6 for mDISC1

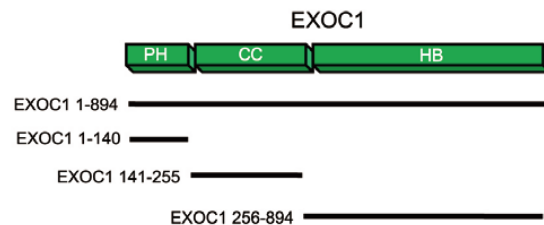
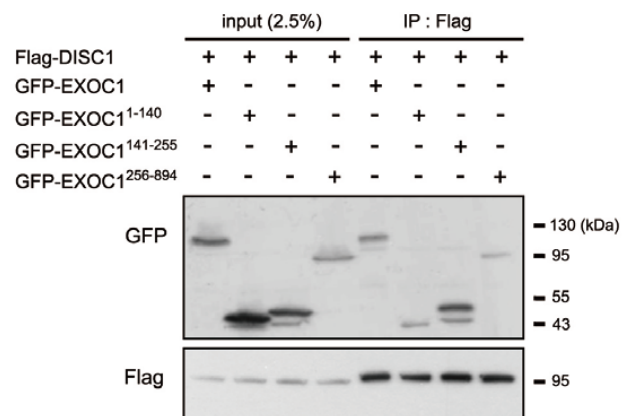
shRNA).

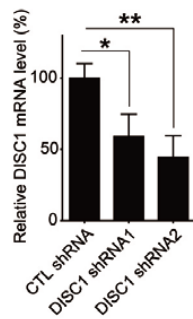
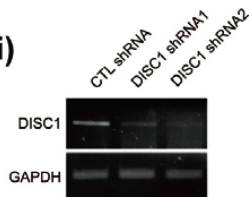
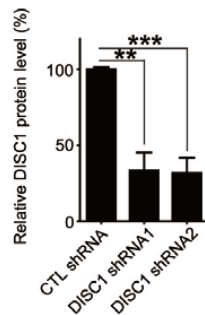
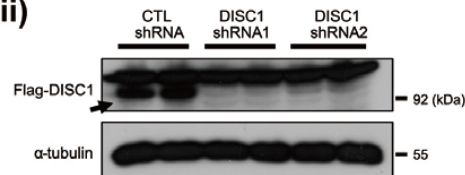
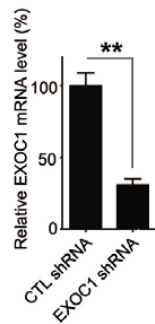
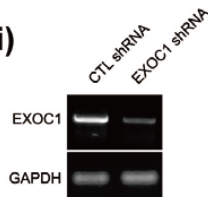
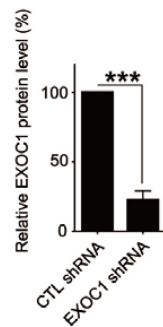
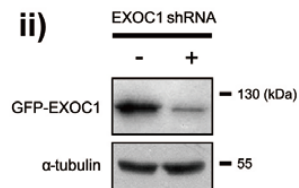
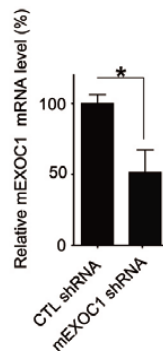
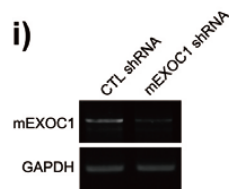
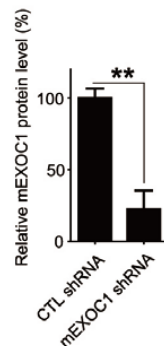
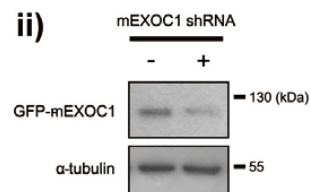
(b) Caffeine-induced ER calcium dynamics in DISC1- or EXOC1-knockdown cells. Average area (*ii*) and amplitudes (*iii*) of the calcium response graph under 100 mM caffeine stimulation (*i*) were statistically analyzed (n= 16 for control shRNA, 25 for EXOC1 shRNA, 19 for DISC1 shRNA, 24 for DISC1 shRNA+EXOC1 shRNA).

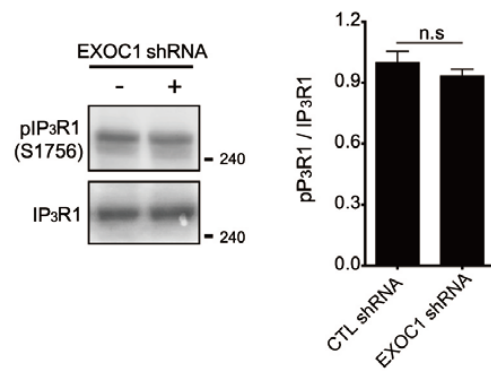
Error bars represent means \pm SEM. Statistical significance was analyzed by two-tailed *t*-test.

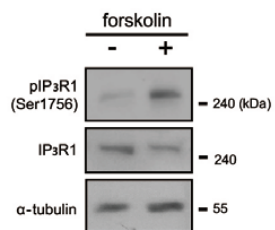
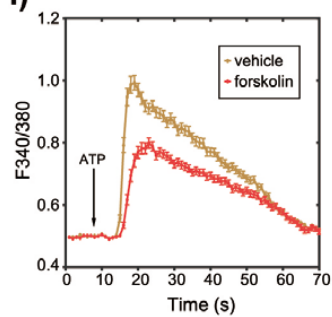
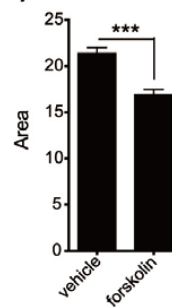
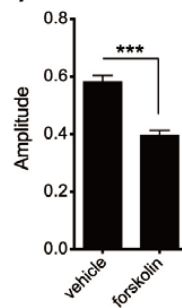
Supplementary Figure 9. Original gel (blot) data in main figures 1-3

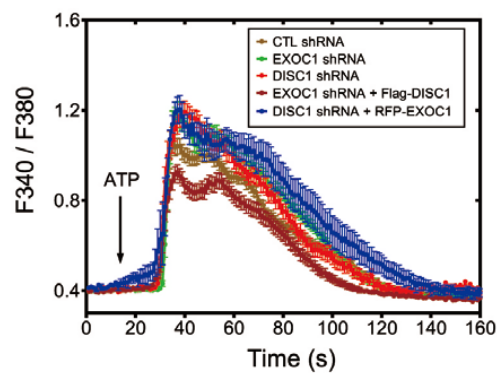
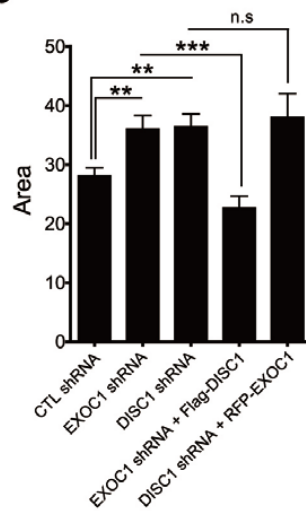
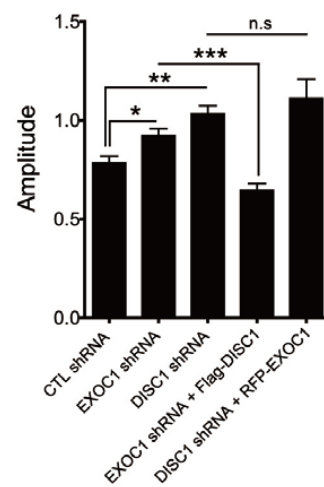
a**i)****ii)****b****i)****ii)****iii)**

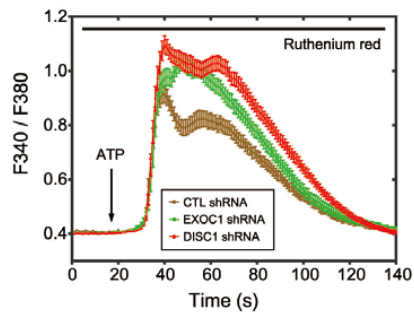
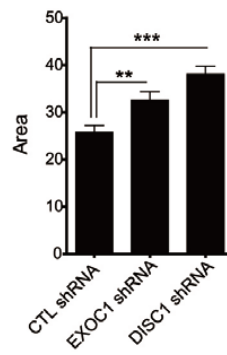
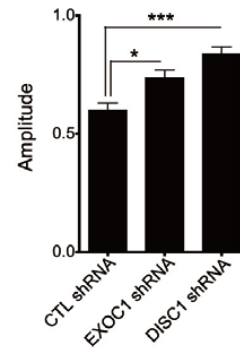
a**b**

a**i)****ii)****b****i)****ii)****c****i)****ii)**



a**b****i)****ii)****iii)**

a**b****c**

a**b****c**

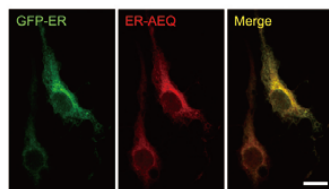
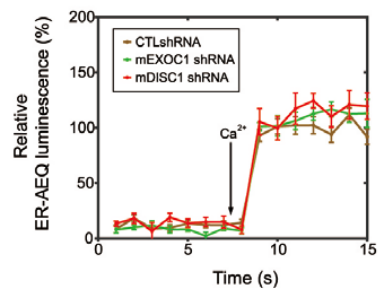
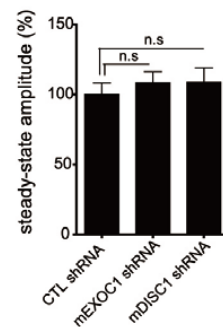
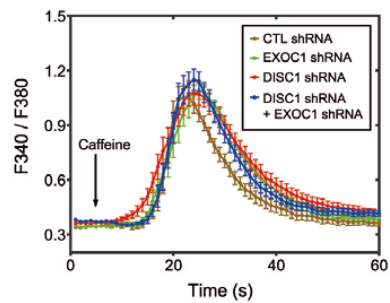
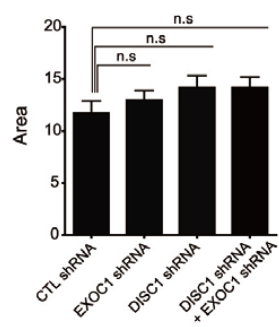
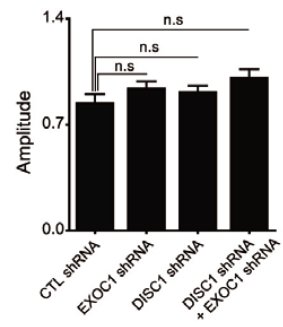
a**i)****ii)****iii)****b****i)****ii)****iii)**

Figure 1ai

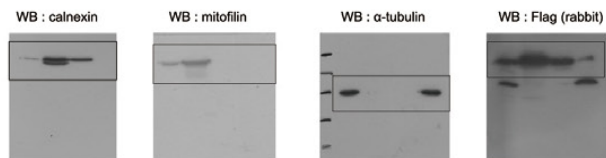


Figure 1aii

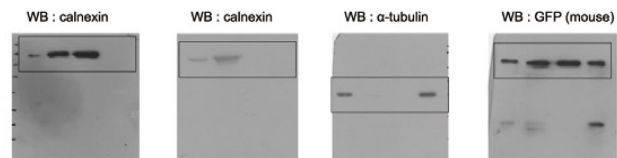


Figure 1aiii

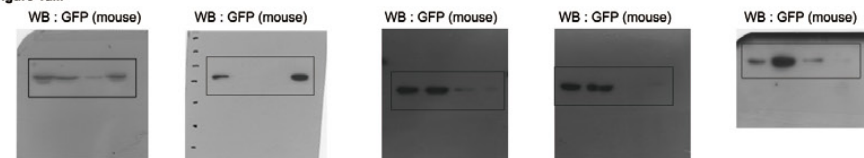


Figure 2a

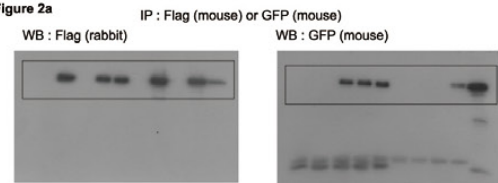


Figure 2c

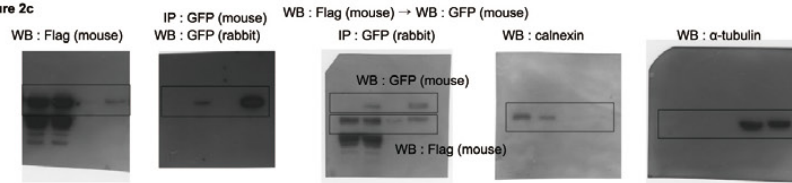


Figure 2d

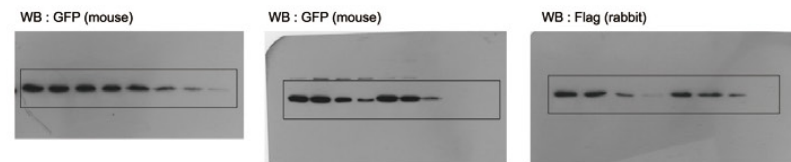


Figure 3a

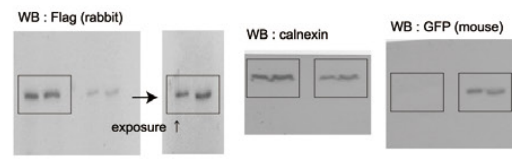


Figure 3b

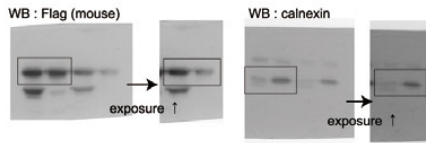


Figure 3c

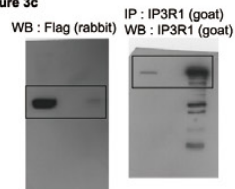


Figure 3d

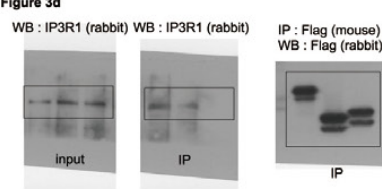


Figure 3e

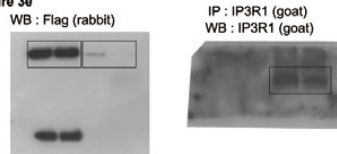


Figure 3f

